REMARKS

Claims 1-74 were pending in this application. Claims 27-74 have been canceled, without prejudice, as being directed to a non-elected invention. Claim 2 has also been canceled, without prejudice, claims 1, 3, 4, 7, 9, 17, 21, 24, 25, and 26 have been amended and new claim 75 has been added. Accordingly, claims 1, 3-26, and 75 will remain pending in the application upon entry of the claim amendments presented herein. For the Examiner's convenience, a copy of the claims that will be pending upon entry of the amendments presented herein is attached hereto as Appendix B.

Support for the amendments to the claims and for new claim 75 may be found throughout the specification, including the originally filed claims. In particular, support for the amendments to claim 1 may be found, for example, at page 19, lines 13-28 of Applicants' specification. *No new matter has been added.*

Attached hereto is Appendix A titled "Version with Markings to Show Changes Made," which indicates the specific amendments made to the specification and the claims.

Amendment of the claims should in no way be construed as an acquiescence to any of the objections/rejections set forth in the instant Office Action, and was done solely to expedite the prosecution of the application. Applicants reserve the right to pursue the claims as originally filed in this or one or more separate applications.

Objection to the Specification

The Examiner has objected to the specification because "it contains an embedded hyperlink and/or other form of browser-executable code."

Applicants have amended the specification to remove all hyperlinks. Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing objection to the specification.

The Examiner also objects to the specification because the specification "has not been checked to the extent necessary to determine the presence of all possible minor errors."

Applicants have reviewed the specification and have corrected minor typographical errors throughout the specification. Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing objection to the specification.

Lastly, the Examiner objects to the specification because

> [t]he use of the trademark ABLAST™, ALGIN™ and GAP™ have been noted in this application. It should be capitalized wherever it appears and be accompanied by the generic terminology. Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

Applicants respectfully traverse the foregoing objection to the specification. Applicants respectfully submit that the use of trademarks having definite meaning is permissible in patent applications (see M.P.E.P. §608.1(v)). Applicants submit that every effort has been made to prevent the use of the trademark in any manner which might adversely affect its validity as a trademark. For example, each trademark is identified by the capitalization of each letter. Moreover, the registered trademark symbol is used to identify each mark as a trademark. Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing objection to the specification.

Rejection of Claims 1-26 Under 35 U.S.C. §112, First Paragraph

The Examiner has rejected claims 1-26 under 35 U.S.C. §112, first paragraph, because, according to the Examiner,

> the specification, while being enabling for a method for identifying a modulator of quorum sensing signaling in bacteria wherein said method comprises: providing a cell which comprises a quorum sensing controlled gene wherein the cell comprises a reporter gene as a means for generating a detectable signal; a contact step; a detection step wherein the amount of B-galactosidase activity is measured and the correlation the measured amount of Beta-galactosidase detected is the detectable signal which would identify the test compound as a modulator of quorum sensing signaling in bacteria does not reasonably provide enablement for a method for identifying a modulator of quorum sensing signaling in bacteria wherein said method comprises: providing a cell which comprises a quorum sensing controlled gene wherein the cell comprises a quorum sensing controlled gene; contacting said cell with a

> quorum sensing signal molecule in the presence and absence of a test compound and detecting a change in the detectable signal to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make or use the invention commensurate in scope with these claims.

In particular, the Examiner is of the opinion that

> [t]here is no teaching within the specification of other means of detection without the use of a [reporter] gene. The specification fails to teach examples of other modes of detecting a change in the detectable signal when there is no requirement for any component within the method to comprise a reporter gene or some mode of detection. Therefore, the specification fails to enable a method for identifying a modulator of quorum sensing signaling in bacteria wherein said method comprises the recited steps. Applicants have provided no guidance to enable one of skill in the art to determine, without undue experimentation, a method for identifying a modulator of quorum sensing signaling in bacteria wherein said method recites that the detectable signal can be measured but does not require any detectable components within the method. If there are no detectable components associated with the cell, then one cannot detect a change; therefore the method is unpredictable, since there are no steps that teach what components are required to create a detectable change. One could not predict whether there would be a change to detect, if the method lacks detectable components.

Applicants respectfully traverse the foregoing rejection for the following reasons. Applicants respectfully submit that the teachings in Applicants' specification clearly enable one of ordinary skill in the art to make and/or use the instant invention using only routine experimentation. Contrary to the Examiner's assertions, the teachings in Applicants' specification are not limited to the use of a reporter gene, *e.g.,* a β-galactosidase gene, to generate a detectable signal. Applicants' specification teaches several methods for generating and detecting a detectable signal in a cell. For example, at page 21, lines 3-23 of the specification, Applicants teach that detecting a change in the detectable signal may be achieved by, for example, detecting alterations in gene transcription of any indicator gene or reporter gene induced upon modulation of quorum sensing signaling or by monitoring: cell viability, the nutritional requirements of a cell, resistance to a drug, *e.g.,* an antibiotic, or global changes to the cell such as changes in second messenger generation. Furthermore, as taught in Applicants'

specification, biofilm formation by the cell as well as increased virulence are additional indicators of increased expression of quorum sending controlled genes (see, for example, page 46, lines 30-36 of the specification). Assays for biofilm formation, signal generation and culture growth are taught in Applicants' specification (see, for example, Example 6, Example 5, and Example 3, respectively). Moreover, as indicated by, for example, Lopez-Lopez, G., *et al.* (1991) *J. Med. Microbiol.* 34(6):349-53; Huchinson, M. L. (1999) *Microbes. Infect.* 1(12):1005-14; Blondeau, J.M. (1998) *Int. J. Antimicrob. Agents* 10(4):297-302; and Davies, D. G. (1998) *Science* 280(5361):295-8, assays for measuring cell viability, resistance to a drug, second messenger generation, increased virulence or biofilm formation were well known in the art at the time of Applicants' invention.

Based on the foregoing teachings in Applicants' specification as well as the general knowledge in the art at the time of Applicants' invention, the ordinarily skilled artisan would be able to make and use the claimed invention using only routine experimentation. Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing rejection.

Rejection of Claims 1-26 Under 35 U.S.C. §112, Second Paragraph

The Examiner has rejected claims 1-26 under 35 U.S.C. §112, second paragraph, as being "indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In particular, the Examiner is of the opinion that

> [c]laim 1 is unclear. It is unclear whether the cell which comprises a quorum sensing controlled gene must posses a means, such as a reporter gene, to allow a detectable signal to be generated or if there is some other way to allow the generation of the detectable signal to occur. Thus, it is unclear whether a detectable signal will be generated. If the cell must comprise a reporter gene to allow a detectable signal to be generated then the cell must also comprise the reporter gene.

Applicants respectfully traverse the foregoing rejection and respectfully submit that claim 1 is clear and definite. Claim 1 is not limited to detection of a detectable signal generated by a reporter gene. Rather, as indicated by the plain language of the claim, claim 1 encompasses the detection of any detectable signal that is generated in response to a quorum sensing signal molecule. As indicated above, Applicants' specification teaches a plethora of such detectable signals that may be used in the methods of the invention. For example, cell viability, resistance

to a drug, second messenger generation, changes in the nutritional requirements of a cell, biofilm formation, and increased virulence of a cell are examples of detectable signals taught in Applicants' specification. In view of the foregoing teachings in Applicants' specification, Applicants respectfully submit that the pending claims are clear and definite. Accordingly, Applicants' respectfully request reconsideration and withdrawal of this rejection under 35 U.S.C. § 112, second paragraph.

Furthermore, the Examiner is of the opinion that

> [c]laim 26 is unclear. It is unclear how to define a modulator that scavenges the quorum sensing signal molecule. Neither the specification nor the claims define "scavenges." Therefore, it is unclear how to define the phrase; as such the metes and bounds of the claim cannot be determined.

Applicants respectfully traverse the foregoing rejection and submit that the term "scavenging" is clearly defined in Applicants' specification as including "the sequestration, chemical modification, or inactivation of a quorum sensing signal molecule such that it is no longer able to regulate quorum sensing gene control" (see page 25, lines 1-7 of the specification). Accordingly, in view of the teachings in Applicants' specification, including the foregoing definition provided in Applicants' specification, Applicants respectfully submit that the term "scavenges" is clear and definite. Accordingly, Applicants respectfully request reconsideration and withdrawal of the foregoing rejection.

Rejection of Claims 1-7, 9-10, 13-15, and 17-26 35 Under 35 U.S.C. §102(b)

The Examiner has rejected claims 1-7, 9-10, 13-15, 17-26 under 35 U.S.C. §102(b) as being anticipated by Pearson *et al.* (1997) *J. of Bacteriol.* 179(18): 5756-5767. In particular, the Examiner is of the opinion that

> Pearson et al., teach the roles of *Pseudomonas aeruginosa las* and *rhl* quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes....The Materials and Method section teaches DNA techniques and the construction of *Pseudomonas aeruginosa lasI* and *lasR* mutants (page 5757). Bioassays for the rhl autoinducer were taught wherein the assay was developed in *E.coli* that contained the constructed plasmid carrying the mutation and a *lacZ* reporter gene wherein the presence or absence of B-galactosidase activity was

measured (page 5758). Thus, a wild type *E.coli* does not express the quorum sensing signal molecule. The E.coli cell comprises a quorum sensing controlled gene and is responsive to a quorum sensing signal molecule such that a detectable signal is generated. The cell comprises a reporter gene is lacZ which is controls B-galactosidase activity, which is the detectable change. In order to identify components of *Pseudomonas aeruginosa* that are necessary and sufficient for expression of the *rhlA* gene, the authors invented a bioassay for quorum sensing control in *E.coli* DH5a by cloning a plasmid that contained an rhlA'lacZ fusion and tacp-rhlR (page 5762). Quorum-sensing control of *rhlA* was shown to require *rhlR* and the production of rhamnolipids was shown to depend on *rhlR* and either *rhll* or PAI-2, which modulated the quorum sensing (page 5762). The system was modulated and allowed the increased expression of *rhlA* by more than 300-fold, however in contrast, the compound PAI-1 has almost no effect on *rhlA* expression (page 5762). Therefore, the inventor concluded that PAI-2 is a modulator of quorum sensing signaling in bacteria (page 5762)....It was found that high concentrations of PAI-1 caused both *lasB* expression and *rhlA* expression to decrease in *E.coli* expressing LasR, these negative effects appear similar to the results of *E.coli* expressing the *V.fischeri* LuxR wherein low levels of VAI-1 activated *luxR* expression and high levels of VAI-1 repressed expression (page 5764). Therefore, when PAI-1 decreased *rhlA* expression, there was an inhibition, which thereby inhibited the cells ability to regulate virulence gene expression, which is the equivalent of inhibiting the host defense mechanism, since the normally produces *rhl* which express components associated with the host defense mechanism.

Applicants respectfully traverse the foregoing rejection under 35 U.S.C. §102(b) for the following reasons.

As amended, claim 1 is directed to a method for identifying a modulator of quorum sensing signaling in bacteria by providing a cell which is capable of endogenously synthesizing a quorum sensing signal molecule, where the cell comprises a regulatory sequence of a quorum sensing controlled gene operatively linked to a gene that generates a detectable signal in response to the quorum sensing signal molecule; contacting the cell with a test compound; and comparing the detectable signal generated in the presence and absence of a test compound, to thereby identify the test compound as a modulator of quorum sensing signaling in bacteria.

New claim 75 is directed to a method for identifying a modulator of quorum sensing signaling in bacteria by providing a cell which comprises a quorum sensing controlled gene

wherein said quorum sensing controlled gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36, operatively linked to a gene that generates a detectable signal in response to a quorum sensing signal molecule, contacting the cell with a quorum sensing signal molecule in the presence and absence of a test compound, and comparing the detectable signal generated in the presence and absence of a test compound to thereby identify the test compound as a modulator of quorum sensing signaling in bacteria.

For a prior art reference to anticipate a claimed invention in terms of 35 U.S.C. §102, the prior art must teach ***each and every element*** of the claimed invention. Lewmar Marine v. Barient, 827 F.2d 744, 3 USPQ2d 1766 (Fed. Cir. 1987).

Applicants respectfully submit that Pearson *et al.* fail to teach each and every limitation of the pending claims for the following reasons. Pearson *et al.* do not teach or suggest a method for identifying a modulator of quorum sensing signaling in bacteria using a cell which is capable of endogenously synthesizing a quorum sensing signal molecule, as is set forth in claim 1. Rather, the Pearson *et al.* bioassay utilizes a *P. aeruginosa lasI rhlI* double-mutant (see page 5762, column 2, last paragraph of the Pearson *et al.* reference). This mutant cannot endogenously produce a quorum sensing signal molecule and therefore the quorum sensing signal molecule must be added exogenously to the bioassay of Pearson *et al.*

Furthermore, Pearson *et al.* do not teach or suggest a method for identifying a modulator of quorum sensing signaling in bacteria using a cell comprising a specific nucleotide sequence selected from the group consisting of SEQ ID NO:1-SEQ ID NO:36, as is set forth in new claim 74. Accordingly, Pearson, *et al.* do not teach or suggest each and every limitation of the claimed invention. For the reasons set forth above, Applicants respectfully request reconsideration and withdrawal of the foregoing rejection under 35 U.S.C. §102(b).

Rejection of Claims 8, 11, 12, and 16 Under 35 U.S.C. §103(a)

The Examiner has rejected claims 8, 11, 12 and 16 under 35 U.S.C. §103(a) as being unpatentable over Pearson *et al.* (1997) J. *of Bacteriol.* 179(18): 5756-5767 in view of Passador *et al.* (1993) *Science* 260:1127-1130. In particular, the Examiner is of the opinion that "Pearson et al., do not teach the method wherein the quorum sensing signal molecule is produced by a second cell." Furthermore, the Examiner is of the opinion that

> Passador et al., teach expression of *P.aeruginosa* virulence genes requires cell-to-cell communication....The experiments show that the *lasI* gene is involved in the synthesis of a diffusible molecule termed *P.aeruginosa* autoinducer (PAI) that provides a means of cell-to-cell communication that is required for the expression of virulence genes (abstract). In this system the expression of *lasB* is monitored by measuring B-galactosidase production from PAO-RI carrying the fusion in the presence of the *lasB::lacZ* or absence of the fusion plasmid (page 1129). The data indicates that Lasl is involved in the production of a diffusible Al-like molecule termed PAI and that both LasR and PAI are required for maximum lasB expression (page 1130) which one cell produces, whereby the other cell can respond to the production of the molecule. The mechanism for cell-cell communication between *P.aeruginosa* cells or between *P.aeruginosa* and other bacterial cells allows the coordinate expression of virulence-associated genes when carried out under the proper environmental conditions (page 1130) whereby two cells communicate, one cell produces the signal molecule while the other cell responds to the molecule. Therefore, it would have been prima facie obvious at the time of applicants invention to modify the method for identifying a modulator of quorum sensing signaling in bacteria comprising the recited steps as taught by Pearson et al., to include a second cell that produces the quorum sensing signal molecule, instead of adding the molecule to the cell. One would have a reasonable expectation of success in using a second cell to produce the molecule since Passador et al., teach that the expression of *P.aeruginosa* virulence genes requires cell-to-cell communication whereby one cell produces the molecule and the other cell can respond to the production of the molecule. Moreover, no more than routine skill would have been required to incorporate a second cell which is a wild type *P.aeruginosa* cell that express one of the virulence factors, using cells constructed in a similar manner as the cells of Pearson et al., which allows detection of a change to identify a compound as a modulator of quorum sensing signaling in bacteria.

Applicants respectfully traverse the foregoing 35 U.S.C. §103 rejection for the following reasons.

To establish a *prima facie* case of obviousness, it is necessary for the Examiner to present evidence, preferably in the form of some teaching, suggestion, incentive or inference in the applied references, or in the form of generally available knowledge, that one having ordinary skill in the art would have been motivated to make the claimed invention and would have had a reasonable expectation of success in making the claimed invention. Under section 103, "[b]oth the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure" (*Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.* 927 F.2d 1200, 1207, 18 USPQ2d 1016 (Fed. Cir. 1991), quoting *In re Dow Chemical Co.,* 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed Cir. 1988)). Moreover, when a combination of references are used to establish a *prima facie* case of obviousness, the Examiner must present evidence that one having ordinary skill in the art would have been motivated to combine the teachings in the applied references in the proposed manner to arrive at the claimed invention. See, *e.g., Carella v. Starlight Archery*, 804 F.2d 135, 231 USPQ 644 (Fed. Cir. 1986); and *Ashland Oil, Inc. v. Delta Resins and Refractories, Inc.*, 776 F.2d 281, 227 USPQ 657 (Fed. Cir. 1985).

As indicated above, the primary reference of Pearson *et al.* fails to teach or suggest a method for identifying a modulator of quorum sensing signaling in bacteria using a cell which is capable of endogenously synthesizing a quorum sensing signal molecule as is required by amended claim 1 and claims depending therefrom. Furthermore, Pearson *et al.* do not teach or suggest a method for identifying a modulator of quorum sensing signaling in bacteria using a cell comprising a specific nucleotide sequence selected from the group consisting of SEQ ID NO:1-SEQ ID NO:36, as is required by new claim 75 and claims depending therefrom.

Moreover, the secondary reference of Passador *et al.* fails to cure the deficiencies in the teachings of the Pearson *et al.* reference. Specifically, Passador *et al.* do not teach or suggest a method for identifying a modulator of quorum sensing signaling in bacteria using a cell which is capable of endogenously synthesizing a quorum sensing signal molecule, as is required by claim 1. Furthermore, Passador *et al.* do not teach or suggest a method for identifying a modulator of quorum sensing signaling in bacteria using a cell comprising a specific nucleotide sequence selected from the group consisting of SEQ ID NO:1-SEQ ID NO:36, as is required by claim 75.

In view of the foregoing, Applicants respectfully submit that the combination of Pearson *et al.* and Passador *et al.* fail to teach or suggest Applicants' invention. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw rejection of the pending claims under 35 U.S.C. §103.

SUMMARY

In view of the above remarks and the amendments to the claims, it is believed that this application is in condition for allowance. If a telephone conversation with Applicants' Attorney would expedite the prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

Respectfully submitted,

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Limited Recognition Under 37 C.F.R. §10.9(b)
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Dated: **March 19, 2003**

APPENDIX A
VERISON WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Please amend the paragraph beginning at page 1, line 3 as follows:

--**Related Applications**

This application claims priority to U.S. Provisional Patent Application No. 60/153,022 filed on September 3, ~~2000~~1999, incorporated herein in its entirety by reference.--

Please amend the paragraph beginning at page 3, line 12 as follows:

-- For example, Cystic fibrosis (CF), the most common inherited lethal disorder in Caucasian populations (~1 out of 2,500 life births), is characterized by bacterial colonization and chronic infections of the lungs. The most prominent bacterium in these infections is *P. aeruginosa*—by their mid-twenties, over 80% of people with CF have *P. aeruginosa* in their lungs (Govan, J. R. *et al.* (1996) *Microbiol Rev.* 60(3):539-74). Although these infections can be controlled for many years by antibiotics, ultimately they "progress to mucoidy," meaning that the *P. aeruginosa* forms a biofilm that is resistant to antibiotic treatment. At this point the prognosis is poor. The median survival age for people with CF is the late 20s, with *P. aeruginosa* being the leading cause of death (Govan, J. R. *et al.* (1996) *Microbiol Rev.* 60(3):539-74). According to the Cystic Fibrosis Foundation, treatment of CF cost more than $900 million in 1995 ~~(Foundation, CF http://www.cff.org/homeline199701.htm)~~.--

Please amend the paragraph beginning at page 4, line 22 as follows:

--Treatment of these so-called nosocomial infections is complicated by the fact that bacteria encountered in hospital settings are often resistant to many antibiotics. In June 1998, the National Nosocomial Infections Surveillance (NNIS) System reported increases in resistance of *P. aeruginosa* isolates from intensive care units of 89 % for quinolone resistance and 32 % for imipenem resistance compared to the years 1993-1997 (see the NNIS webiste, ~~http://www.cdc.gov/ncidod/hip/NNIS/AR_Surv1198.htm~~). In fact, some strains of *P. aeruginosa* are resistant to over 100 antibiotics (Levy, S. (1998) *Scientific American.* March). There is a critical need to overcome the emergence of bacterial strains that are resistant to conventional antibiotics (Travis, J. (1994) *Science.* 264:360-362).--

Please amend the paragraph beginning at page 5, line 28 as follows:

-- In general, the invention pertains to the modulation of bacterial cell-to-cell signaling. The inhibition of quorum sensing signaling renders a bacterial population more susceptible to treatment, either directly through the host immune-response or in combination with traditional antibacterial agents and biocides. More particularly, the invention also pertains to a method for ~~identifing~~ identifying modulators, *e.g.*, inhibitors of cell-to-cell signaling in bacteria, and in particular one particular human pathogen, *Pseudomonas aeruginosa*.--

Please amend the paragraph beginning at page 8, line 34 as follows:

-- In another embodiment, the invention is an isolated nucleic acid molecule comprising ~~a~~ a polynucleotide that hybridizes under stringent conditions to a quorum sensing controlled genetic locus derived from the genome of *Pseudomonas aeruginosa*, wherein the genetic locus comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36, operatively linked to a reporter gene.--

Please amend the paragraph beginning at page 9, line 20 as follows:

--and detecting a change in the detectable signal to thereby identify the test compound as a modulator of quorum sensing signaling in bacteria.--

Please amend the paragraph beginning at page 10, line 13 as follows:

-- *Figure 1* depicts the ~~paragdigm~~ paradigm for quorum sensing signaling in the target bacterium, *Pseudomonas aeruginosa*.--

Please amend the paragraph beginning at page 13, line 10 as follows:

--The invention is based on the interruption of bacterial cell-to-cell ~~singaling~~ signaling, *i.e.*, quorum sensing signaling in order to render a bacterial population more susceptible to treatment, either through the host immune-response or in combination with traditional antibacterial agents and biocides. Thus, the invention provides a bacterial indicator strain that allows for a high throughput screening assay for identifying compounds that modulate, *e.g.*, inhibit bacterial cell-to-cell ~~signalling~~ signaling. The compounds so identified will provide novel anti-pathogenics and anti-fouling agents.--

Please amend the paragraph beginning at page 13, line 21 as follows:

--The term "analog" as in "homoserine lactone analog" is intended to encompass compounds that are chemically and/or electronically similar but have different atoms, such as isosteres and isologs. An analog includes a compound with a structure similar to that of another compound but differing from it in respect to certain components or structural makeup. The term analog is also ~~intened~~ intended to encompass stereoisomers.--

Please amend the paragraph beginning at page 16, line 1 as follows:

-- Autoinducer synthase molecules can be obtained from naturally occurring sources, *e.g.*, by purifying cellular extracts, can be chemically synthesized or can be recombinantly produced. Recombinantly produced autoinducer synthase molecules can have the amino acid sequence of ~~a~~ a naturally occurring form of the autoinducer synthase protein. They can also have a similar amino acid sequence which includes mutations such as substitutions and deletions (including truncation) of a naturally occurring form of the protein. Autoinducer synthase molecules can also include molecules which are structurally similar to the structures of naturally occurring autoinducer synthase proteins, *e.g.*, biologically active variants.--

Please amend the paragraph beginning at page 17, line 1 as follows:

--The terms "derived from" or "derivative", as used interchangeably herein, are intended to mean that a sequence is identical to or modified from another sequence, *e.g.*, a naturally occurring sequence. Derivatives within the scope of the invention include polynucleotide derivatives. Polynucleotide or nucleic acid derivatives differ from the sequences described herein (*e.g.*, SEQ ID Nos.: 1-38) or known in nucleotide sequence. For example, a polynucleotide derivative may be characterized by one or more nucleotide substitutions, insertions, or deletions, as compared to a reference sequence. A nucleotide sequence comprising a quorum sensing controlled genetic locus that is derived from the genome of *P. aeruginosa*, *e.g.*, SEQ ID Nos.: 1-38, includes sequences that have been modified by various changes such as insertions, deletions and substitutions, and which retain the property of being regulated in response to a quorum sensing signaling event. Such sequences may comprise a quorum sensing controlled regulatory element and/or a quorum sensing controlled gene. The nucleotide sequence of the *P. aeruginosa* genome is available at the Pseudomonas Genome Project website ~~www.pseudomonas.com~~.--

Please amend the paragraph beginning at page 21, line 24 as follows:

--As used interchangeably herein, the terms "transposon" and "transposable element" are intended to include a piece of DNA that can insert into and cut itself out of, genomic DNA of a particular host species. Transposons include mobile genetic elements (MGEs) containing insertion sequences and additional genetic sequences unrelated to insertion functions (for example, sequences encoding a reporter gene). Insertion sequence elements include sequences that are between 0.7 and 1.8 kb in size with termini approximately 10 to 40 base pairs in length with perfect or nearly perfect repeats. As used herein, a transposable element is operatively linked to the nucleotide sequence into which it is inserted. Transposable elements are well known in the art~~, and are described for example, at www.bact.wisc.edu/MicrotextBook/BactGenetics~~.--

Please amend the paragraph beginning at page 22, line 19 as follows:

-- Quorum sensing signal molecules that are useful in the methods of the present invention include autoinducer compounds such as homoserine lactones, and analogs thereof (see Table 1). In certain embodiments, the quorum sensing signal molecule is either 3-oxo-C12-homoserine lactone or C4-HSL. In one embodiment, the cell does not express the quorum sensing signal molecule. For example, the cell may comprise a mutant strain of *Pseudomonas aeruginosa* wherein *lasI* and *rhlI* are inactivated. Therefore, the cell is contacted with an exogenous quorum sensing signal molecule, *e.g.*, a recombinant or synthetic molecule. In another embodiment, the quorum sensing signal molecule is produced by a second cell (*e.g.*, a prokaryotic or eukaryotic cell), which is co-incubated with the indicator cell. For example, an indicator cell which does not express a quorum sensing signal molecule can be co-incubated with a wild type strain of *Pseudomonas aeruginosa* which produces a quorum sensing signal molecule. Alternatively, the indicator strain which does not express a quorum sensing signal molecule is co-incubated with a second cell which has been transformed, or otherwise altered, such that it is able to express a quorum sensing signal ~~moleucle~~ molecule. In yet another embodiment, the quorum sensing signal molecule is expressed by the indicator strain.--

Please amend the paragraph beginning at page 26, line 4 as follows:

-- In one embodiment, the cell is contacted with an exogenous quorum sensing signal molecule, *e.g.*, a recombinant or synthetic molecule, as described herein. In another embodiment, the quorum sensing signal molecule is produced by a second cell (*e.g.*, a prokaryotic or eukaryotic cell), which is co-incubated with the indicator cell. For example, an indicator cell which does not express a quorum sensing signal molecule can be co-incubated with a wild type strain of *Pseudomonas aeruginosa* which produces a quorum sensing signal molecule. Alternatively, the indicator strain which does not express a quorum sensing signal

molecule is co-incubated with a second cell which has been transformed, or otherwise altered, such that it is able to express a quorum sensing signal ~~moleucle~~ molecule. In yet another embodiment, the quorum sensing signal molecule is expressed by the indicator strain.--

Please amend the paragraph beginning at page 26, line 15 as follows:

-- Another aspect of the invention provides a mutant strain of *Pseudomonas aeruginosa* comprising a promoterless reporter gene inserted in a ~~chromsome~~ chromosome at a genetic locus comprising a nucleotide sequence set forth as SEQ ID NOs:1-36, *e.g.*, a quorum sensing controlled genetic locus. In one embodiment the reporter gene is contained in a transposable element. In another embodiment, the reporter gene is lacZ or GFP, or a variant thereof, *e.g.*, GFPmut2. In yet another embodiment, *lasI* and *rhlI* are inactivated in the mutant strain of *P. aeruginosa*. The above-described cells are useful in the methods of the instant invention, as the cells are responsive to a quorum sensing signal molecule such that a detectable signal is generated by the reporter gene. These cells are also useful for studying the function of polypeptides encoded by the quorum sensing controlled loci comprising the nucleotide sequences set forth as SEQ ID NOs.:1-36.--

Please amend the paragraph beginning at page 26, line 27 as follows:

-- Yet another aspect of the invention provides isolated nucleic acid molecules comprising a nucleotide sequence comprising a quorum sensing controlled genetic locus derived from the genome of *Pseudomonas aeruginosa* operatively linked to a reporter gene. In one embodiment, a ~~reproter~~ reporter gene is operatively linked to a regulatory sequence derived from the genome of *P. aeruginosa*, wherein the regulatory sequence regulates a quorum sensing controlled genetic locus comprising a nucleotide sequence set forth as SEQ ID NO:1-36. In a preferred embodiment such regulatory sequences comprise at least one binding site for a quorum sensing controlled transcriptional regulatory factor (*e.g.*, a transcriptional activator or repressor molecule) such that transcription of the reporter gene is responsive to a quorum sensing ~~singal~~ signal molecule and/or a modulator of quorum sensing signaling. In another embodiment, a reporter gene is operatively linked to a quorum sensing controlled genetic locus derived from the genome of *P. aeruginosa*, wherein the genetic locus comprises a nucleotide sequence set forth as SEQ ID NO:1-36. In yet another embodiment, a reporter gene is operatively linked to a nucleotide sequence which has at least 80%, and more preferably at least 85%, 90% or 95% identity to quorum sensing controlled genetic locus derived from the genome of *P. aeruginosa*, wherein the genetic locus comprises a nucleotide sequence set forth as SEQ ID NO:1-36. In a further embodiment, a reporter gene is operatively linked to a nucleotide sequence which hybridizes under stringent conditions to quorum sensing controlled genetic locus derived from

the genome of *P. aeruginosa*, wherein the genetic locus comprises a nucleotide sequence set forth as SEQ ID NO:1-36.--

Please amend the paragraph beginning at page 27, line 10 as follows:

-- The term "isolated nucleic acid molecule" includes nucleic acid molecules which are separated from other nucleic acid molecules which are present in the natural source of the nucleic acid. For example, with regard to genomic DNA, the term "isolated" includes nucleic acid molecules which are separated from the chromosome with which the genomic DNA is naturally associated. Preferably, an "isolated" nucleic acid is free of sequences which naturally flank the nucleic acid (*i.e.*, sequences located at the 5' and 3' ends of the nucleic acid) in the genomic DNA of the organism from which the nucleic acid is derived. For example, in various embodiments, the isolated nucleic acid molecule can contain less than about 5 kb, 4kb, 3kb, 2kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized. As used interchangeably herein, the terms "nucleic acid molecule" and "polynucleotide" are intended to include DNA molecules (*e.g.*, cDNA or genomic DNA) and RNA molecules (*e.g.*, mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA. The term "DNA" refers to deoxyribonucleic acid whether single- or double-stranded. As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which include an open reading frame encoding a protein, preferably a quorum sensing controlled protein, and can further include non-coding regulatory sequences, and introns.--

Please amend the paragraph beginning at page 27, line 33 as follows:

--The present invention includes polynucleotides capable of hybridizing under stringent conditions, ~~prefereably~~ preferably highly stringent conditions, to the polynucleotides described herein (*e.g.*, a quorum sensing controlled genetic locus, *e.g.*, SEQ ID NOs.:1-36). As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences that are significantly identical or homologous to each other remain hybridized to each other. Preferably, the conditions are such that sequences at least about 70%, more preferably at least about 80%, even more preferably at least about 85% or 90% identical to each other remain hybridized to each other. Such stringent conditions are known to those skilled in the art and can be found in *Current Protocols in*

Molecular Biology, Ausubel *et al.*, eds., John Wiley & Sons, Inc. (1995), sections 2, 4, and 6. Additional stringent conditions can be found in *Molecular Cloning: A Laboratory Manual*, Sambrook *et al.*, Cold Spring Harbor Press, Cold Spring Harbor, NY (1989), chapters 7, 9, and 11. A preferred, non-limiting example of stringent hybridization conditions includes hybridization in 4X sodium chloride/sodium citrate (SSC), at about 65-70°C (or alternatively hybridization in 4X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 1X SSC, at about 65-70°C. A preferred, non-limiting example of highly stringent hybridization conditions includes hybridization in 1X SSC, at about 65-70°C (or alternatively hybridization in 1X SSC plus 50% formamide at about 42-50°C) followed by one or more washes in 0.3X SSC, at about 65-70°C. A preferred, non-limiting example of reduced stringency hybridization conditions includes hybridization in 4X SSC, at about 50-60°C (or alternatively hybridization in 6X SSC plus 50% formamide at about 40-45°C) followed by one or more washes in 2X SSC, at about 50-60°C. Ranges intermediate to the above-recited values, *e.g.*, at 65-70°C or at 42-50°C are also intended to be encompassed by the present invention. SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1X SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes each after hybridization is complete. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, $T_m(°C) = 2(\#\text{ of A + T bases}) + 4(\#\text{ of G + C bases})$. For hybrids between 18 and 49 base pairs in length, $T_m(°C) = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1X SSC = 0.165 M). It will also be recognized by the skilled practitioner that additional reagents may be added to hybridization and/or wash buffers to decrease non-specific hybridization of nucleic acid molecules to membranes, for example, nitrocellulose or nylon membranes, including but not limited to blocking agents (*e.g.*, BSA or salmon or herring sperm carrier DNA), detergents (*e.g.*, SDS), chelating agents (*e.g.*, EDTA), Ficoll, PVP and the like. When using nylon membranes, in particular, an additional preferred, non-limiting example of stringent hybridization conditions is hybridization in 0.25-0.5M NaH_2PO_4, 7% SDS at about 65°C, followed by one or more washes at 0.02M NaH_2PO_4, 1% SDS at 65°C (see *e.g.*, Church and Gilbert (1984) *Proc. Natl. Acad. Sci. USA* 81:1991-1995), or alternatively 0.2X SSC, 1% SDS.--

Please amend the paragraph beginning at page 29, line 3 as follows:

-- The invention further encompasses nucleic acid molecules that differ from the quorum sensing controlled genetic loci described herein, *e.g.*, the nucleotide sequences shown in SEQ ID

NO:1-36. Accordingly, the invention also includes variants, *e.g.*, allelic variants, of the disclosed polynucleotides or proteins; that is naturally occuring and non-naturally ~~occuring~~ occurring alternative forms of the isolated polynucleotide which may also encode proteins which are identical, homologous or related to that encoded by the polynucleotides of the invention.--

Please amend the paragraph beginning at page 29, line 36 as follows:

--The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (*J. Mol. Biol.* (48):444-453 (1970)) algorithm which has been incorporated into the GAP™ program in the GCG™ software package (available at ~~http://www.gcg.com~~ the ACCELRYS™ website), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the GAP™ program in the GCG™ software package (available at ~~http://www.gcg.com~~ the ACCELRYS™ website), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity between two amino acid or nucleotide sequences is determined using the algorithm of E. Meyers and W. Miller (*Comput. Appl. Biosci.*, 4:11-17 (1988) which has been incorporated into the ALIGN™ program (version 2.0) (available at ~~http://vega.igh.cnrs.fr/bin/align-guess.cgi~~ the ALIGN™ website), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.--

Please amend the paragraph beginning at page 30, line 14 as follows:

-- The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST™ and XBLAST™ programs (version 2.0) of Altschul, *et al.* (1990) *J. Mol. Biol.* 215:403-10. BLAST™ nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to nucleic acid molecules of the invention. BLAST™ protein searches can be performed with the XBLAST™ program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST™ can be utilized as described in Altschul *et al.*, (1997) *Nucleic Acids Res.* 25(17):3389-3402. When utilizing BLAST™ and Gapped BLAST™ programs, the default parameters of the respective programs (*e.g.*, XBLAST™ and NBLAST™) can be used. See ~~http://www.ncbi.nlm.nih.gov~~ the

National Center for Biotechnology website. Additionally, the "Clustal" method (Higgins and Sharp, Gene, 73:237-44, 1988) and "Megalign" program (Clewley and Arnold, *Methods Mol. Biol*, 70:119-29, 1997) can be used to align sequences and determine similarity, identity, or homology.--

Please amend the paragraph beginning at page 32, line 18 as follows:

-- As used interchangeably herein, a "cell" or a "host cell" includes any cultivatable cell that can be modified by the introduction of heterologous DNA. As used herein, "heterologous DNA", a "heterologous gene" or "heterologous polynucleotide sequence" is defined in relation to the cell or organism harboring such a nucleic acid or gene. A heterologous DNA sequence includes a sequence that is not naturally found in the host cell or organism, *e.g.*, a sequence which is native to a cell type or species of organism other than the host cell or organism. Heterologous DNA also includes mutated endogenous genetic ~~seqeunces~~ sequences, for example, as such sequences are not naturally found in the host cell or organism. Preferably, a host cell is one in which a quorum sensing signal molecule, *e.g*, an autoinducer molecule, initiates a quorum sensing signaling response which includes the regulation of target quorum sensing controlled genetic sequences . The choice of an appropriate host cell will also be influenced by the choice of detection signal. For example, reporter constructs, as described herein, can provide a selectable or screenable trait upon activation or inhibition of gene transcription in response to a quorum sensing signaling event; in order to achieve optimal selection or screening, the host cell phenotype will be considered.--

Please amend the paragraph beginning at page 35, line 37 as follows:

--3.1-kb *P. aeruginosa* PAO1 chromosomal DNA fragment containing the *lasB* gene was amplified by PCR using the ~~Expand~~EXPAND™ Long Template PCR System (Boehringer Mannheim). This fragment was cloned into *Bam*HI-digested pSUP102. The resulting plasmid, pSUP102-lasB was digested with *Not*I, polished with T4 polymerase and ligated with the 6.5-kb *lacZ-aacC1* fragment from pTL61T-GMΩ1 to generate pMW300. The promoterless *lacZ* gene in pMW300 is 549 nucleotides form the start of the *lasB* ORF, it is flanked by 1.5 kb upstream and 1.6 kb downstream *P. aeruginosa* DNA, and it contains the p15A *ori,* which does not support replication in *P. aeruginosa*.--

Please amend the paragraph beginning at page 36, line 26 as follows:

--Southern Blotting. Chromosomal DNA was prepared using the QIAMP™ tissue kit (Qiagen Inc.). Approximately 2 μg of chromosomal DNA was digested with restriction endonucleases, separated on a 0.7% agarose gel, and transferred to a nylon membrane according

to standard methods (Ausubel, F. *et al.* (1997) *Short Protocols in Molecular Biology.* (John Wiley & Sons, Inc., New York, N.Y.). DNA probes were generated using digoxigenin-11-dUTP by random primed DNA labeling or PCR. The Southern blots were visualized using the ~~Genius~~GENIUS™ system as outlined by the manufacturer (Boehringer Mannheim).--

Please amend the paragraph beginning at page 38, line 4 as follows:

--DNA sequences flanking Tn5-B22 insertions were located on the *P. aeruginosa* PAO1 chromosome by searching the chromosomal database at the *P. aeruginosa* Genome Project web site ~~(www.pseudomonas.com)~~. The ORFs containing the insertions are those described at the web site. Functional coupling from the Argonne National Labs WIT website ~~(http://wit.mcs.anl.gov/WIT2)~~, sequence analysis, and expression patterns of the qsc mutants were used to identify potential operons (Overbeek, R. *et al.* (1999) *PNAS* 96, 2896-2901).--

Please amend the paragraph beginning at page 38, line 13 as follows:

--Identification of *Pseudomonas aeruginosa* qsc Genes. Seven thousand Tn5::B22 mutants of *P. aeruginosa* PAO-MW1 were screened. Tn5::B22 contains a promoterless *lacZ*. *P. aeruginosa* PAO-MW1 is a *lasI, rhlI* mutant that does not make acyl-HSL signals. Thus, transcription of the Tn5::B22 *lacZ* in a qsc gene was expected to respond to an acyl-HSL signal. The screen involved growth of each mutant in a complex medium in a microtiter dish well with no added acyl-HSL, $3OC_{12}$-HSL, C_4-HSL, or both $3OC_{12}$-HSL and C_4-HSL. After 12-16 hours, β-galactosidase activity in each culture was measured. Two hundred-seventy mutants showed greater than_2 fold stimulation of β-galactosidase activity in response to either or both acyl-HSL. Of these, 70 showed a greater than_5-fold stimulation of β-galactosidase activity in response to either or both acyl-HSL, and were studied further. Each mutant was grown with shaking in culture tubes and 47 showed a reproducible greater than_5-fold stimulation of β-galactosidase activity in response to either or both of the acyl-HSL signals. These were considered to have Tn5::B22 insertions in qsc genes. It was shown by a Southern blot analysis with a *lacZ* probe that each mutant contained a single Tn5::B22 insertion.--

Please amend the paragraph beginning at page 39, line 26 as follows:

--Identity and Analysis of qsc Genes. The Tn5-B22-marked qsc genes were identified by coupling arbitrary PCR or transposon cloning with DNA sequencing. The sequences were located in the *P. aeruginosa* PAO1 chromosome by searching the *Pseudomonas aeruginosa* Genome Project web site (the Pseudomonas Genome Project website ~~www.pseudomonas.com~~). To confirm the locations of the Tn5-B22 insertions in each qsc mutant, a Southern blot analysis was performed with Tn5-B22 as a probe. The sizes of Tn5-B22 restriction fragments were in

agreement with those predicted based on the *P. aeruginosa* genomic DNA sequence (data not shown). The 47 qsc mutations mapped in or adjacent to 39 different open reading frames (ORFs). For example Figure 3 depicts the nucleic acid sequence of the quorum sensing controlled locus on the *P. aeruginosa* chromosome mapped in the *P. aeruginosa* mutant strain qsc102.--

In the Claims:

Please cancel claims 2 and 27-74, without prejudice, and amend claims 1, 3, 4, 7, 9, 17, 21, 24, 25, and 26 as follows:

1. **(Amended)** A method for identifying a modulator of quorum sensing signaling in bacteria, said method comprising:

providing a cell which is capable of endogenously synthesizing a quorum sensing signal molecule, wherein said cell ~~which~~ comprises a regulatory sequence of a quorum sensing controlled gene operatively linked to a gene that generates a detectable signal in response to the quorum sensing signal molecule; ~~wherein said cell is responsive to a quorum sensing signal molecule such that a detectable signal is generated;~~

contacting said cell with ~~a quorum sensing signal molecule in the presence and absence of~~ a test compound;

and comparing ~~detecting a change in~~ the detectable signal generated in the presence and absence of a test compound, to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria.

3. **(Amended)** The method of claim ~~2~~ 1 or 75, wherein said ~~signal generation means comprises a reporter gene, and wherein said quorum sensing signal molecule causes transcription of said reporter gene, said transcription providing said detectable signal~~ gene that generates a detectable signal comprises a reporter gene that is heterologous to said regulatory sequence.

4. **(Amended)** The method of claim 3, wherein said detectable signal is provided by the transcription of said reporter gene or the translation product of said reporter gene.

7. **(Amended)** The method of claim 1 or 75, wherein said cell does not express said quorum sensing signal molecule.

9. **(Amended)** The method of claim ~~1~~75, wherein said cell is a prokaryote or eukaryote.

17. **(Amended)** The method of claim 1 or 75, wherein said quorum sensing controlled gene is endogenous to said cell.

21. **(Amended)** The method of claim 1 or 75, wherein said quorum sensing signal molecule is an autoinducer of said quorum sensing controlled gene.

24. **(Amended)** The method of claim 1 or 75, wherein the test compound modulates quorum sensing signaling by inhibiting a bacterial enzyme involved in the synthesis of said quorum sensing signal molecule.

25. **(Amended)** The method of claim 1 or 75, wherein the test compound modulates quorum sensing signaling by inhibiting bacterial reception of said quorum sensing signal molecule.

26. **(Amended)** The method of claim 1 or 75, wherein the test compound modulates quorum sensing signaling by scavenging said quorum sensing signal molecule.

Please add new claim 75 as follows:

75. **(New)** A method for identifying a modulator of quorum sensing signaling in bacteria, said method comprising:

providing a cell which comprises a quorum sensing controlled gene wherein said quorum sensing controlled gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36, operatively linked to a gene that generates a detectable signal in response to a quorum sensing signal molecule;

contacting said cell with a quorum sensing signal molecule in the presence and absence of a test compound;

and comparing the detectable signal generated in the presence and absence of a test compound to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria.

APPENDIX B

1. A method for identifying a modulator of quorum sensing signaling in bacteria, said method comprising:

providing a cell which is capable of endogenously synthesizing a quorum sensing signal molecule, wherein said cell comprises a regulatory sequence of a quorum sensing controlled gene operatively linked to a gene that generates a detectable signal in response to the quorum sensing signal molecule;

contacting said cell with a test compound;

and comparing the detectable signal generated in the presence and absence of a test compound, to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria.

3. The method of claim 1 or 75, wherein said gene that generates a detectable signal comprises a reporter gene that is heterologous to said regulatory sequence.

4. The method of claim 3, wherein said detectable signal is provided by the transcription of said reporter gene or the translation product of said reporter gene.

5. The method of claim 4, wherein said reporter gene is selected from the group consisting of *ADE1, ADE2, ADE3, ADE4, ADE5, ADE7, ADE8, ASP3, ARG1, ARG3, ARG4, ARG5, ARG6, ARG8, ARO2, ARO7, BAR1, CAT, CHO1, CYS3, GAL1, GAL7, GAL10, GFP, HIS1, HIS3, HIS4, HIS5, HOM3, HOM6, ILV1, ILV2, ILV5, INO1, INO2, INO4, lacZ, LEU1, LEU2, LEU4, luciferase, LYS2, MAL, MEL, MET2, MET3, MET4, MET8, MET9, MET14, MET16, MET19, OLE1, PHO5, PRO1, PRO3, THR1, THR4, TRP1, TRP2, TRP3, TRP4, TRP5, URA1, URA2, URA3, URA4, URA5* and *URA10*.

6. The method of claim 5, wherein said reporter gene is *lacZ* or *GFP*.

7. The method of claims 1 or 75, wherein said cell does not express said quorum sensing signal molecule.

8. The method of claim 7, wherein said quorum sensing signal molecule is produced by a second cell.

9. The method of claim 75, wherein said cell is a prokaryote or eukaryote.

10. The method of claim 9, wherein said cell is a bacterium.

11. The method of claim 8, wherein said second cell is a prokaryote or eukaryote.

12. The method of claim 11, wherein said second cell is a bacterium.

13. The method of claim 10 or 12, wherein said bacterium is a gram negative bacterium.

14. The method of claim 13, wherein said gram negative bacterium is *Pseudomonas aeruginosa*.

15. The method of claim 10, wherein said bacterium is a mutant strain of *Pseudomonas aeruginosa* which comprises a regulatory sequence of a quorum sensing controlled gene operatively linked to a reporter gene, wherein in said mutant strain, *lasI* and *rhlI* are inactivated.

16. The method of claim 12, wherein said second cell is wild type *Pseudomonas aeruginosa*.

17. The method of claims 1 or 75, wherein said quorum sensing controlled gene is endogenous to said cell.

18. The method of claim 10, wherein said quorum sensing controlled gene encodes a virulence factor.

19. The method of claim 10, wherein said quorum sensing controlled gene encodes a polypeptide which inhibits a bacterial host defense mechanism.

20. The method of claim 10, wherein said quorum sensing controlled gene encodes a polypeptide which regulates biofilm formation.

21. The method of claim 1 or 75, wherein said quorum sensing signal molecule is an autoinducer of said quorum sensing controlled gene.

22. The method of claim 21, wherein said autoinducer is a homoserine lactone.

23. The method of claim 22, wherein said test compound is a homoserine lactone analog.

24. The method of claim 1 or 75, wherein said modulator inhibits an enzyme involved in the synthesis by said bacterium of said quorum sensing signal molecule.

25. The method of claim 1 or 75, wherein said modulator inhibits reception of said quorum sensing signal molecule by said bacterium.

26. The method of claim 1 or 75, wherein said modulator scavenges said quorum sensing signal molecule.

75. A method for identifying a modulator of quorum sensing signaling in bacteria, said method comprising:

providing a cell which comprises a quorum sensing controlled gene wherein said quorum sensing controlled gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36, operatively linked to a gene that generates a detectable signal in response to a quorum sensing signal molecule;

contacting said cell with a quorum sensing signal molecule in the presence and absence of a test compound;

and comparing the detectable signal generated in the presence and absence of a test compound to thereby identify said test compound as a modulator of quorum sensing signaling in bacteria.

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